

# Chromosomal assignment of AFLP markers in upland cotton (*Gossypium hirsutum* L.)

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Received: 26 May 2008 / Accepted: 2 September 2008 / Published online: 4 November 2008  
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**Abstract** In this research, we used two sets of cotton aneuploid (*G. hirsutum* × *G. tomentosum* and *G. hirsutum* × *G. barbadense*) plants to locate AFLP markers to chromosomes using deletion analysis method. Thirty-eight primer combinations were used to generate 608 polymorphic AFLP markers. A total of 98 AFLP markers were assigned to 22 different cotton chromosomes or chromosome arms. Of those assigned markers, 63.3% were assigned to A genome and 36.7% were assigned to D genome. A low rate (14.3%) of common markers were found between those assigned AFLP markers with the AFLP markers from an intraspecific cross population developed previous in our lab. Based on the 16 common markers, we were able to associate the 13 linkage groups previously identified in our lab to eight chromosomes. Further research will be carried out by using SSR markers with known location to associate unassigned linkage groups to chromosomes.

**Keywords** AFLP · *G. hirsutum* · QTL · Chromosomal assignment

## Abbreviation

AFLP Amplified fragment length polymorphism

Cotton (*Gossypium* spp.) is the world's leading fiber crop and an important source of oil as well. The genus *Gossypium* L. comprises 50 diploid and tetraploid species. Among the four cultivated *Gossypium* species in the world, the American allotetraploid species (*Gossypium hirsutum* L. and *Gossypium barbadense* L.) dominate worldwide cotton production, having almost displaced the old-world diploid cultivars (*Gossypium arboreum* L. and *Gossypium herbaceum* L.) (Lee 1984). Diploid species of the genus *Gossypium* fall into eight different genome types designated A–G and K (Percival et al. 1999). All tetraploid species are allopolyploids and probably derive from a single A × D polyploidization event (Endrizzi et al. 1985). Variation in ploidy among *Gossypium* spp., together with a tolerance for aneuploidy in tetraploid cotton species, has facilitated the use of cytogenetic techniques to explore cotton genetics and evolution research. The 26 chromosomes of the tetraploid cotton genome have arbitrarily been numbered 1–13 and 14–26 for the A- and D-related subgenomic groups based on pairing relationships in diploid × tetraploid crosses (Kimber 1961), respectively. Among 198 mutants identified in cotton, 61 mutant loci have been assembled into 16 linkage groups, 11 of which have

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been associated with chromosomes using monosomic and monotelodisomic stocks (Endrizzi et al. 1985). Also, aneuploid substitution stocks have been used to assign individual RFLP (Reinisch et al. 1994; Ulloa et al. 2005) and SSR (Liu et al. 2000) markers to chromosomes or chromosome arms, allowing the assignment of linkage groups to chromosomes.

Amplified fragment length polymorphism (AFLP) is a DNA fingerprinting technique capable of detecting several loci in a single PCR reaction (Zabeau and Vos 1993; Vos et al. 1995). This method is based on the selective amplification of restriction enzyme digested fragments from genomic DNA using different primer combinations. In comparison to other methods, the AFLP method generates virtually unlimited numbers of DNA fragments from nanogram quantities of genomic DNA. The AFLP technique also uses very stringent PCR conditions which provide a better reproducibility in comparison to other PCR-based marker systems. The AFLP method combines the reliability of RFLPs and the power and sensitivity of PCR-based methods. However, AFLP method normally provides dominant markers. This method can be used in combination with other types of markers to quickly develop highly saturated linkage maps in plant species and is especially useful for crops with large genomes like cotton (*Gossypium* spp., 4700 cM). The AFLPs have been used for QTL mapping studies in many crops, including rice (Maheswaran et al. 1997), barley (Becker et al. 1995; Powell et al. 1997), and oat (Jin et al. 1998), in other crops (Hansen et al. 1999; Shan et al. 1999) as well as in cotton (Lacape et al. 2003; Mei et al. 2004).

A genetic map is necessary not only for the reliable detection, mapping, and estimation of gene effects of important agronomic traits, but also for further research on the structure, organization, evolution, and function of the plant genome. Restriction fragment length polymorphism (RFLP) maps of allotetraploid cotton have been constructed from both interspecific (Reinisch et al. 1994; Wright et al. 1999; Saranga et al. 2001) and intraspecific (Shappley et al. 1996, 1998; Ulloa and Meredith 2000; Ulloa et al. 2002) mapping populations. Of the 705 RFLP loci mapped to 41 linkage groups in the interspecific *Gossypium* populations, the actual chromosome identity of only 14 of the linkage groups was presented (Reinisch et al. 1994). A combined RFLP–SSR–AFLP map of tetraploid cotton based on a *G. hirsutum* × *G. barbadense*

backcross population was recently reported (Lacape et al. 2003). The map consists of 888 loci, including 465 AFLPs, 229 SSRs, 192 RFLPs, and two morphological markers, ordered in 37 linkage groups. Recently, a more saturated genetic map constructed using 3,347 markers loci was reported (Rong et al. 2004). In all of these genetic maps, aneuploid stocks were employed to locate markers to individual chromosomes and identify linkage groups to chromosomes. In cotton, monotelodisomic stocks that are hemizygous for one arm provide an easy means to localize genes and marker loci to one arm or the other of a given chromosome (Endrizzi et al. 1985; Saha and Stelly 1994). Assignment of RFLP and SSR markers to chromosomes have been reported by Reinisch et al. (1994) and Liu et al. (2000), respectively. Very few reports are available on the assignment of AFLP markers to chromosomes in cotton (Lacape et al. 2003). Here we report our results on the assignment of the AFLP markers to chromosomes in cotton.

## Materials and methods

AFLP markers were assigned to cotton chromosomes and chromosome arms following the method described by Lazo et al. (1994) for dominant DNA markers. Two sets of aneuploid genetic stock were used in this research viz., genetic stocks, monosomic for *G. tomentosum* chromosomes 1, 2, 6, 7, 9, 10, 16, 17, 18, 20, and 25 and stocks monotelodisomic for 1Lo, 1Sh, 2Lo, 2Sh, 3Lo, 3Sh, 4Lo, 4Sh, 5Lo, 6Lo, 6Sh, 7Sh, 8Lo, 9Lo, 10Lo, 10Sh, 11Lo, 12Lo, 14Lo, 15Lo, 16Lo, 17Sh, 18Lo, 18Sh, 20Lo, 20Sh, 22Lo, 22Sh, 25Lo, 26Lo, and 26Sh. In addition, genetic stocks monosomic for *G. barbadense* chromosomes 4, 6, 9, 10, 12, 17, 18, 20, 13, and 26 and monotelodisomic for 2Sh, 3Sh, 4Lo, 4Sh, 5Sh, 7Lo, 7Sh, 8Sh, 9Sh, 11Sh, 14Sh, 16Lo, 16Sh, 17Lo, 18Lo, 18Sh, 20Lo, 20Sh, 25Sh, 26Lo, and 26Sh were also considered (Table 1). The markers were assigned to chromosomes or chromosome arms based on the screening of individual monosomic or monotelodisomic F<sub>1</sub> plants. In each F<sub>1</sub>, the “donor genotype” is euploid *G. tomentosum* or *G. barbadense* and the “recipient genotype” is hypoaneuploid *G. hirsutum* (TM1). TM1 is an inbred line derived from “Deltapine 14” and is considered the genetic standard of Upland cotton (*G. hirsutum*) (Kohel et al. 1970). A monosomic F<sub>1</sub> substitution stock has a single chromosome from the donor

**Table 1** Cotton aneuploid genetic stocks

<i>G. hirsutum</i> × <i>G. barbadense</i> (BC <sub>0</sub> F <sub>1</sub> )		
Monosomic (missing whole chromosome)		4, 6, 9, 10, 12, 17, 18, 20, 23, 26
Monotelodisomic (missing short arm)		2 <i>Sh</i> , 3 <i>Sh</i> , 4 <i>Sh</i> , 5 <i>Sh</i> , 7 <i>Sh</i> , 8 <i>Sh</i> , 9 <i>Sh</i> , 11 <i>Sh</i> , 14 <i>Sh</i> , 16 <i>Sh</i> , 18 <i>Sh</i> , 20 <i>Sh</i> , 25 <i>Sh</i> , 26 <i>Sh</i>
Monotelodisomic (missing long arm)		4 <i>Lo</i> , 7 <i>Lo</i> , 16 <i>Lo</i> , 17 <i>Lo</i> , 18 <i>Lo</i> , 20 <i>Lo</i> , 26 <i>Lo</i>
<i>G. hirsutum</i> × <i>G. tomentosum</i> (BC <sub>0</sub> F <sub>1</sub> )		
Monosomic (missing whole chromosome)		1, 2, 6, 7, 9, 10, 16, 17, 18, 20, 25
Monotelodisomic (missing short arm)		1 <i>Sh</i> , 2 <i>Sh</i> , 3 <i>Sh</i> , 4 <i>Sh</i> , 5 <i>Sh</i> , 6 <i>Sh</i> , 7 <i>Sh</i> , 9 <i>Sh</i> , 10 <i>Sh</i> , 11 <i>Sh</i> , 12 <i>Sh</i> , 14 <i>Sh</i> , 15 <i>Sh</i> , 16 <i>Sh</i> , 18 <i>Sh</i> , 20 <i>Sh</i> , 22 <i>Sh</i> , 25 <i>Sh</i> , 26 <i>Sh</i>
Monotelodisomic (missing long arm)		1 <i>Lo</i> , 2 <i>Lo</i> , 3 <i>Lo</i> , 4 <i>Lo</i> , 6 <i>Lo</i> , 8 <i>Lo</i> , 17 <i>Lo</i> , 18 <i>Lo</i> , 20 <i>Lo</i> , 22 <i>Lo</i> , 26 <i>Lo</i>

substituted for the corresponding chromosome *pair* of the recipient genotype. Similarly, monotelodisomic F<sub>1</sub> stocks lack alleles from the recurrent parent in the hemizygous chromosome arm from the donor, but carry alleles of the recurrent parent on the opposing arm (either in homozygous or heterozygous condition, depending on the patterns of crossing over). An intrahirsutum cross involving Paymaster × PeeDee 2135 with as many as 138 F<sub>2,3</sub> progenies were used for development of a linkage map using AFLP markers (Akash 2003). The so far developed linkage map data covering 1773.2 cM (37.7% of cotton genome) by placing 38 primer combinations was used in the present study. Thirty-eight primer combinations were selected in the present investigation for chromosomal assignment.

#### DNA isolation

DNA was isolated from young leaves of individual plants from the aneuploid genetic stocks. The DNA-easy Plant Mini Kit (Qiagen, Santa Clarita, CA) was used to extract DNA. Fresh young leaves (0.5 mg) were ground in liquid nitrogen and used to extract DNA. The detail protocol was as described in the manufacturer's instructions. An agarose gel method was used to provide information regarding both DNA quantity and quality. The concentration of genomic DNA was estimated by comparing the size and intensity of each sample band with those of a sizing standard, DNA mass ladder (Invitrogen, Carlsbad, CA). The DNA samples were diluted to a concentration of 20 ng/μl with TE<sub>0.1</sub> (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) to be used as a working solution in AFLP marker analysis.

#### AFLP analysis

A total of 38 primer combinations were used to generate AFLP data (Table 2). The procedure was followed according to Vos et al. (1995) with some modifications. Sample DNA was digested with *Eco*RI and *Mse*I restriction enzymes and oligonucleotide adapters specific to enzyme restriction sites were ligated to the resulting fragments through incubation (150 min, 37°C) with DNA ligase. This step was carried out in GeneAmp PCR System 9600 (Perkin-Elmer). The pre-amplification was carried out according to Vos et al. (1995) and selective amplification was performed using a Li-Cor (Lincoln, NE) AFLP selective amplification kit. Electrophoresis was conducted on an automatic DNA sequencer (Li-Cor 4200 series DNA sequencer). Amplified DNA fragments were separated on a 6% denaturing polyacrylamide gel. The real-time TIFF images were automatically collected and recorded during electrophoresis (Fig. 1). The gel images were automatically scored by Li-Cor Saga Generation 2 software with GT & MX modules client version 3.1.0 build 315. The nomenclature of AFLP loci was according to Lacape et al. 2003.

#### Results

##### AFLP marker frequency in cotton

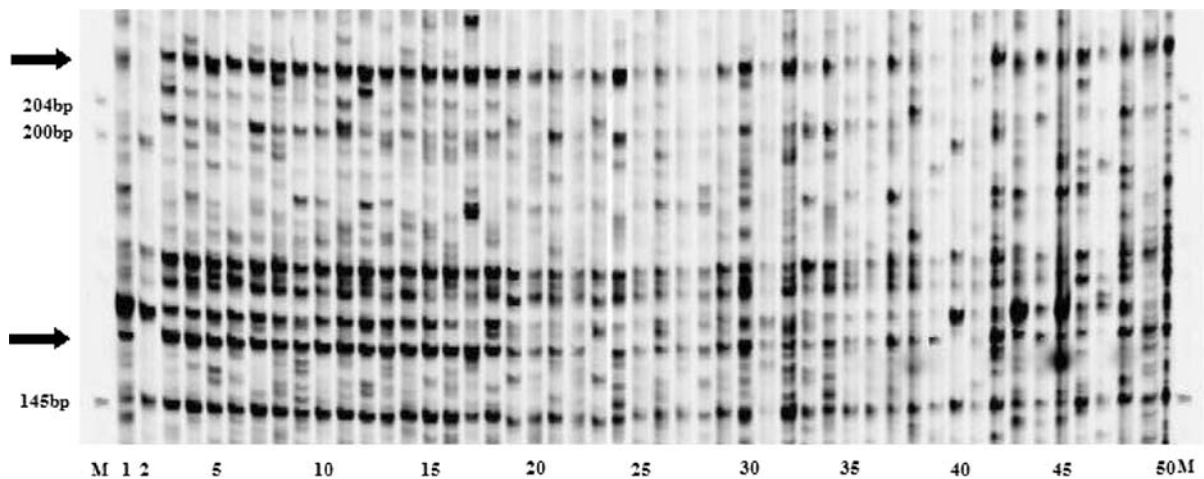
Thirty-eight primer combinations were used in this research. A total of 3,421 and 3,371 major AFLP bands were observed for the two sets of aneuploids, *G. hirsutum* × *G. tomentosum* and *G. hirsutum* × *G.*

**Table 2** AFLP polymorphic frequency in cotton aneuploid genetic stocks

Name	Primer pairs		<i>G. hirsutum</i> × <i>G. tomentosum</i>			<i>G. hirsutum</i> × <i>G. barbadense</i>		
	<i>EcoRI</i>	<i>MseI</i>	Total	Polymorphic	Percent	Total	Polymorphic	Percent
E1M1	AAC	CAA	102	10	9.8	98	7	7.1
E1M2	AAC	CAC	75	12	16.0	76	8	10.5
E1M4	AAC	CAT	102	5	4.9	97	5	5.2
E1M5	AAC	CTA	84	16	19.0	82	8	9.8
E2M1	AAG	CAA	106	8	7.5	102	10	9.8
E2M5	AAG	CTA	116	13	11.2	116	11	9.5
E2M7	AAG	CTG	86	9	10.5	87	7	8.0
E2M8	AAG	CTT	72	11	15.3	65	6	9.2
E3M1	ACA	CAA	103	11	10.7	97	9	9.3
E3M3	ACA	CAG	112	15	13.4	118	12	10.2
E3M4	ACA	CAT	102	9	8.8	96	5	5.2
E3M5	ACA	CTA	58	8	13.8	61	5	8.2
E3M6	ACA	CTC	71	5	7.0	84	7	8.3
E3M7	ACA	CTG	82	4	4.9	77	5	6.5
E5M2	ACC	CAC	92	9	9.8	90	6	6.7
E5M3	ACC	CAG	63	4	6.3	68	4	5.9
E5M4	ACC	CAT	85	11	12.9	80	6	7.5
E5M5	ACC	CTA	126	10	7.9	125	10	8.0
E5M6	ACC	CTC	106	14	13.2	101	13	12.9
E5M8	ACC	CTT	135	16	11.9	138	14	10.1
E6M1	ACG	CAA	102	10	9.8	107	7	6.5
E6M2	ACG	CAC	105	5	4.8	105	8	7.6
E6M4	ACG	CAT	96	6	6.3	95	4	4.2
E6M6	ACG	CTC	120	11	9.2	122	9	7.4
E6M7	ACG	CTG	98	9	9.2	105	5	4.8
E6M8	ACG	CTT	106	13	12.3	102	9	8.8
E4M1	ACT	CAA	62	7	11.3	49	4	8.2
E4M3	ACT	CAG	102	6	5.9	108	8	7.4
E4M4	ACT	CAT	66	8	12.1	63	5	7.9
E4M6	ACT	CTC	57	4	7.0	54	6	11.1
E4M7	ACT	CTG	62	3	4.8	64	3	4.7
E4M8	ACT	CTT	84	12	14.3	82	10	12.2
E7M2	AGC	CAC	75	8	10.7	70	8	11.4
E7M3	AGC	CAG	55	4	7.3	44	3	6.8
E7M4	AGC	CAT	92	7	7.6	95	6	6.3
E7M5	AGC	CTA	114	9	7.9	111	11	9.9
E8M1	AGG	CAA	52	3	5.8	58	6	10.3
E8M5	AGG	CTA	95	6	6.3	79	7	8.9
Total			3421	331	9.7	3371	277	8.2
Average			90.0	8.71	9.7	88.7	7.29	8.2

*barbadense*, respectively (Table 2). A total of 331 and 277 polymorphic bands were observed in those two sets. The polymorphism rate (9.7%) of *G.*

*hirsutum* × *G. tomentosum* is slightly higher than the rate (8.2%) of *G. hirsutum* × *G. barbadense*. The level of polymorphism is not significantly different among



**Fig. 1** AFLP gel image for the primer pair combination *EcoRI* + *ACA/MseI* + *CAA*. The DNA samples are: (from left to right) standard size marker, TM1, *G. tomentosum*, Te12Lo, H17, Te18Lo, H25, Te1Lo, Te17Sh, Te26Sh, Te22Lo, Te16Lo, H18, Te25Lo, Te20Sh, H16, Te15Lo, Te22Sh, Te20Lo, Te26Lo, Te14Lo, Te5Lo, Te6Sh, Te8Lo, Te4Sh, Te7Sh, Te3Sh, H20, Te4Lo, H7, Te3Lo, Te18Sh, Te5Lo, Te6Sh, Te2Sh, Te2Lo, Pima 3-79\*\*TM1, H9, H10, H2, TM1, Te9Lo, Te1Sh, *G. tomentosum*, NTN12-11, Te10Sh, H1, Pima 3-97, NTN17-11, Te10Lo, Te1Lo,

standard size marker. Marker E3M1\_159 (*bottom arrow*) shows polymorphism between TM1 and *G. tomentosum*, and all aneuploid samples present that band except Te7Sh (line 23) indicated that Marker E3M1\_159 is located on short arm of chromosome 7. The Marker E3M1\_207 (*upper arrow*) shows polymorphism between TM1 and *G. tomentosum*, while all the aneuploid F1 stock have that band indicated that Marker E3M1\_207 are likely located on chromosomes where aneuploid were not available

these crosses. *G. tomentosum* is endemic to Hawaii and more closely allied with *G. hirsutum* than with *G. barbadense* (Fryxell 1979; Wendel et al. 1995). The number of bands generated by individual primer combinations ranged from 44 for E7M3 (*EcoRI* + *AGC/MseI* + *CAG*) to 138 for E5M8 (*EcoRI* + *ACC/MseI* + *CTT*), with a mean of 88.7 bands for *G. hirsutum* × *G. barbadense* aneuploid, and 90 bands for the *G. hirsutum* × *G. tomentosum* aneuploid. The primer combination *EcoRI* + *ACC/MseI* + *CTT* produced the largest number of polymorphic products (16 in total) and the primer combination *EcoRI* + *AAC/MseI* + *CTA* had the largest polymorphic rate (19.0%). The polymorphism level detected in this study conforms to the results of Lacape et al. (2003) (11.3%). Also this result is similar to polymorphism revealed in other crops by AFLP: barley (11%) (Becker et al. 1995), and soybean (7.8%) (Young et al. 1999).

#### Assignment of AFLP markers to chromosomes

Since AFLP markers are dominant and the monosomic lines were developed in a TM-1 background, only the polymorphic AFLP markers present in TM-1 and

absent in the aneuploid series of *G. tomentosum* or *G. barbadense* were assigned to a chromosome or chromosome arm. The monosomic lines provided the ability to assign markers to a chromosome, while monotelodisomic lines were used to associate the marker with the short or long arm of a chromosome, and the results were further confirmed using the monosomic lines where available. In this research, 61 markers were assigned to 16 different chromosomes from the analysis of the aneuploid series ( $BC_0F_1$ ) of *G. hirsutum* × *G. tomentosum* (Table 3) and 52 markers were assigned to 17 different chromosomes from the analysis of *G. hirsutum* × *G. barbadense* aneuploid plants ( $BC_0F_1$ ). Of those assigned AFLP markers, 15 were common markers in these two sets of aneuploids. Of these 98 markers, 15 markers were assigned to whole chromosomes, and 83 markers were assigned to different chromosome arms. The number of markers assigned to each chromosome varied from 1 (chromosome 6) to 12 (chromosomes 2). A total of 62 markers (63.3%) were located on the A genome (chromosomes 1–13) and 36 markers (36.7%) were located on the D genome (chromosomes 14–26). This observation is consistent with the results of Lacape et al. (2003)

**Table 3** AFLP markers and their chromosome location

<i>G. hirsutum</i> × <i>G. tomentosum</i>		Common markers		<i>G. hirsutum</i> × <i>G. barbadense</i>	
Markers	Location	Markers	Location	Markers	Location
E3M6_204	1Lo	E5M6_252	2Sh	E2M1_536	2Lo
E4M1_97	1Lo	E6M4_128	2Sh	E2M5_134	2Lo
E2M1_164	2Lo	E3M5_102	3Sh	E6M2_175	2Lo
E3M1_312	2Lo	E1M5_119	7Sh	E7M2_168	2Lo
E3M6_111	2Lo	E4M3_20	7Sh	E1M2_450	3Lo
E3M7_78	2Lo	E3M6_64	9Sh	E2M7_310	3Lo
E2M8_56	2Sh	E4M6_51	9Sh	E4M4_217	3Lo
E3M1_221	2Sh	E4M6_278	10	E2M1_129	4Lo
E5M4_102	3Lo	E2M8_71	10	E6M1_203	4Sh
E1M5_187	4Lo	E3M1_259	10	E1M4_95	5Lo
E1M5_51	4Lo	E2M8_112	12Sh	E6M7_209	5Lo
E5M2_310	4Lo	E1M5_53	17Sh	E1M5_332	6
E8M1_45	4Sh	E3M6_260	25Lo	E3M3_156	7Lo
E3M5_142	5Lo	E8M1_300	26Sh	E4M3_320	7Lo
E3M7_175	5Lo	E7M2_166	26Sh	E3M4_95	7Sh
E3M1_159	7Sh			E6M6_96	7Sh
E3M7_270	7Sh			E6M8_320	7Sh
E5M4_86	7Sh			E3M7_118	10
E1M2_238	9			E6M8_246	10
E3M1_141	9Lo			E4M6_46	12
E4M1_78	9Lo			E1M5_146	12
E7M3_292	9Lo			E1M5_135	12
E8M1_170	9Lo			E7M2_84	12
E3M6_64	9Lo			E4M6_48	11Lo
E1M4_136	10Lo			E3M5_68	14Lo
E2M8_96	10Sh			E5M4_321	16Sh
E3M7_51	10Sh			E7M3_135	17Sh
E3M1_292	14Lo			E5M5_201	18Lo
E1M4_193	15Lo			E5M2_324	18Lo
E8M1_78	15Lo			E5M8_79	18Sh
E1M5_86	17Sh			E1M2_64	20Lo
E2M1_80	17Sh			E2M8_112	20Lo
E1M4_70	17Sh			E6M2_175	20Sh
E3M1_154	18Lo			E6M4_128	23
E3M6_86	18Sh			E3M7_361	23
E3M6_89	18Sh			E4M6_46	23
E6M2_79	18Sh			E4M1_152	26Sh
E5M3_64	22Sh				
E4M1_169	22Sh				
E7M4_309	25				
E3M5_125	25Lo				
E5M4_57	25Lo				
E1M5_69	26Lo				
E3M1_179	26Lo				
E3M1_85	26Lo				
E1M5_154	26Sh				

(64 and 34%, respectively, on the A and D genomes). Of these 98 markers, 15 were in common with the markers of Akash (2003) in the intraspecific *G. hirsutum* population Paymaster 54 × PeeDee 2165 and 21 were in common with those in a ‘Guazuncho-2’ × ‘VH8-4602’ population, which is an interspecific cross of *G. hirsutum* × *G. barbadense* (Lacape et al. 2003).

Not all of the polymorphic markers identified between TM1 and *G. tomentosum* or *G. barbadense* could be assigned to either a chromosome or a chromosome arm. Since both aneuploid series are incomplete, as more aneuploid stocks are developed the potential exists for locating those markers to a chromosome.

#### Association of linkage groups to chromosomes

Based on those assigned markers, we were able to assign our previously developed AFLP linkage map (Akash 2003) to chromosomes (Table 4). Fifteen common markers were detected in the two sets of AFLP makers. Based on those common markers, linkage group 15 and linkage group 5 were associated with chromosome 10; linkage group 3 and linkage group 28 were associated to chromosome 15 long arm; linkage groups 13 and 21 were assigned to chromosome 12; linkage group 4 and 10 were assigned to chromosome 2 and linkage groups 1, 9, 16, 18 and 23 were assigned to chromosomes 17, 7, 22, and 26, respectively (Table 4). There were two common markers on linkage group

1(E1M5\_86 and E1M5\_53), linkage group 4 (E2M1\_536 and E6M2\_175) and linkage group 5 (E3M7\_51 and E4M7\_47), which confirms their chromosome assignment. In this research, we were unable to associate the remaining 15 groups to chromosomes because of lack of common markers.

## Discussion

#### Association of AFLP markers to chromosomes

In this research, 98 polymorphic AFLP markers were assigned to cotton chromosomes or chromosome arms using aneuploid genetic stocks. However, the remaining polymorphic AFLP markers could not be assigned to a cotton chromosome. Possible reasons for this are as follows: (1) the aneuploid genetic stock for the specific chromosome where the marker was located was not available; we are missing aneuploids for chromosomes 13, 19, 21, and 24; (2) only polymorphic markers present in *G. hirsutum* and absent in *G. tomentosum* can be associated with chromosomes using aneuploid genetic stocks and (3) because AFLP markers are dominant, the assignment is based on presence or absence of a specific band. Sometimes scoring the bands was difficult. The assigned AFLP markers were scattered over the various cotton chromosomes with no apparent clustering pattern. The AFLP loci are highly represented among the A subgenome (63.3%) linkage groups when compared to the D subgenomes linkage groups (36.7%). The marker distribution pattern observed is consistent with that of Lacape et al. (2003) in an interspecific cross and with Zhao et al. (1995) with respect to differences in repetitive DNA content between A and D subgenomes. At least one AFLP marker was assigned to each of 22 different cotton chromosomes and 83 markers were localized to different chromosome arms. Recently several papers were published on the chromosomal location of SSR markers in cotton (Liu et al. 2000; Nguyen et al. 2004; Zhang et al. 2002; Song et al. 2005). However, very few reports are available on the chromosomal location of AFLP markers (Lacape et al. 2003, 2005).

#### Association of linkage groups to chromosomes

A chromosome-specific molecular mapping strategy will be very useful for developing a high resolution

**Table 4** Results of assignment of linkage groups to chromosomes

Linkage group	Reference marker	Chromosome location
LG1	E1M5_86,E1M5_53	17Sh
LG3	E1M4_193	15Lo
LG4	E2M1_536, E6M2_175	2Lo
LG5	E3M7_51, E4M7_47	10Sh
LG9	E1M5_119	7Lo
LG10	E2M8_56	2Sh
LG13	E7M2_84	12
LG15	E2M8_71	10
LG16	E5M3_64	22Sh
LG18	E3M7_361	23
LG21	E4M6_46	12
LG23	E1M5_154	26Sh
LG28	E3M4_78	15Lo

consensus map of molecular markers. It will also aid in germplasm introgression efforts that utilize chromosome substitution lines and in map-based chromosome walking and cloning of desirable genes in cotton. The outcome of assigning AFLP markers to chromosomes will be their utility in helping to develop a more complete chromosome specific molecular map of cotton. We were able to assign 14 linkage groups to their corresponding chromosomes in this research. With the low frequency (14.3%) of common AFLP markers found between the aneuploid stocks and the intraspecific cross of the *G. hirsutum* cultivar Paymaster 54 and the germplasm line PeeDee 2165 previously developed in our lab, we were unable to assign all linkage groups to chromosomes. Further work will be needed to locate those unassigned linkage groups to chromosomes.

**Acknowledgment** We thank Dr. D.M. Stelly, Soil and Crop Science Department of Texas A&M University for developing and providing the aneuploid cytogenetic stocks for this study.

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